REMARKS

Claims 1-24 are pending and were all rejected under various grounds as discussed below.

Applicants thank the Examiner for his consideration and withdrawal of a large number of objections and rejections under § 112 and § 103(a) in view of Applicants amendments and remarks in their prior response. These will not be discussed again here for the sake of brevity.

The maintained and new grounds for rejection will be discussed below.

In brief, Applicants are amending claims 1 and 24 in several ways:

(a) the method is now defined as one of determining the relative copy number (rather than just "copy number").

(b) the relationship of NucSeqI and NucSeqI' and the relationship of NucSeqI and NucSeqII' are introduced into the body of the claim and taken from the definitions provided in the specification.

(c) as a purely voluntary amendment, the phrase " wherein the sample comprises a chromosome-derived second nucleotide sequence II (NucSeqII)" is being transposed from part (2) to part (1) of the claim, where it reads more logically.

(d) other cosmetic changes are made to improve the clarity, one example being the replacement of "NucSeqI is amplified" with "amplifying NucSeqI".

Applicants note that due to the amendment of claim 1 to insert the definition of NucSeqI, NucSeqI', *etc.*, , the "identities" of *NucSeqI* with *NucSeqI'* in claim 20 and *NucSeqII* with *NucSeqII'* in claim 21 are now "possible," logical and clear and definite. The definition states that *NucSeq*I hybridizes to the complement of *NucSeq*I' (under stringent conditions) so that *NucSeq*I can be identical with *NucSeq*I'. The same relationship is true for *NucSeq*II and *NucSeq*II'.

None of these amendments introduce new matter and their entry "after final" is respectfully requested since the amendments have narrowed the issues and placed the claims in better condition for allowance or for appeal.

I. OBJECTION TO SPECIFICATION

Examiner acknowledged Applicant's correction of certain registered trademarks by insertion of the registered symbol, but noted that the registered trademarks were not capitalized. Correction was required.

Applicants' Response

The specification has been amended as shown above to rectify this oversight, so that this ground for objection may be withdrawn

II. MAINTAINED REJECTIONS UNDER 35 U.S.C. § 112, Second Paragraph

A. The rejection of claims 1, 3-5, 8-9, and 11-15 rejection for indefiniteness (Item 17 in Action) was maintained because claim 1 allegedly fails to recite a step "of determining the copy number of a first nucleotide sequence."

Applicants Response to A

Claims 1 (and 24) are amended to read: "method of determining the **relative** copy number (CN)...". It is believed that this should be adequate to render the claims definite.

B. The rejection of claims 7-9 for indefiniteness (Item 18 in Action was maintained for the following reasons. The term "NucSeqI' " in claim 1 is allegedly used by the claim to mean "corresponding to NucSeqI", while the accepted meaning is "complementary to NucSeqI". The uncommon definition is not clearly defined in the specification....

Applicants Response to B

The specification defines the relevant terms at page 3, line 35 to pg 4, line 5, as follows (emphasis added)

> With respect to the term "corresponding" as used in the present invention in conjunction with nucleotide sequences, this is intended to mean that the nucleotide sequences I and I' (and II and II'), or more specifically the nucleotide sequence of one and the complementary sequence of the other, are capable of hybridizing under stringent conditions. If the sequences I and I' (and II and II') do not have the same length, the shortest of the two is preferably at most 50% shorter, more preferably at most 30% shorter.

In amended claims 1 and 24, the above definition is introduced. Applicant believe that this definition is stated somewhat more explicitly and clearly in the claims, but it certainly fits within what the application defines in the section cited above (and is not new matter).

C. The rejection of Claims 10-16 for indefiniteness (Item 19 in the Action) was maintained for the following reasons. The term "NucSeqII' " in claim 1 is said to be used by the claim to mean "corresponding to NucSeqII", while the accepted meaning is "complementary to NucSeqII". The uncommon definition is not clearly defined in the specification...

Applicants' Response to C

As noted in the section immediately above, the amendments to claim 1 (and 24) introduces a clearer definition into the claims which should cure any potential indefiniteness in dependent claims 10-16.

III. NEW REJECTIONS UNDER § 112/Second Paragraph

A. New claims 17-23 allegedly omit essential steps (item 20 in Action), namely, determining the copy number of a first nucleotide sequence as recited in the preamble of claim 1.

Applicants' Response to A

As noted in section IA, above, the amendment to claim 1 (and 24) to read: "method of determining the relative copy number (CN)..." is believed to render these claims definite.

B. New claim 24 **omits essential steps (item** 21 in Action), namely determining the copy number of a first nucleotide sequence as recited in the preamble of claim 24.

Applicants' Response to B

As noted in section IA and IIA, above, the amendment to claim 24 to read: "method of determining the relative copy number (CN)..." is believed to render this claims definite.

C. Claims 17-23 and 24 (item 22 in Action), were rejected because the terms "NucSeqI'" and "NucSeqII'" in claims 1 and 24 are allegedly used by to mean "corresponding to NucSeqI" and "corresponding to NucSeqII" respectively, while the accepted meaning is "complementary to NucSeqI" and "complementary to NucSeqII" respectively. The uncommon definitions are not clearly defined in the specification.

Applicants' Response to C

The Examiner is respectfully directed to the discussion above in Sections IB and IC. Introduction of definitions into claims 1 and 24 provide the necessary meaning to the indicated terms, thereby rendering the rejected independent and dependent claims definite.

D. Claims 1-24 (Item 23 in Action) recite "monitored by fluorescence" but allegedly do not provide a **sufficient antecedent basis** rendering the monitoring of the amplification indefinite. Subsequent dependent claims 2, 6, 7, 9, 10, and 13-16 are also considered indefinite. Because, according to the Action, i**t is unclear from where the fluorescence in the claims**

originates. There is no recitation of which, nucleotides, primers, polymerases and probes if any, is fluorescently labeled.

Applicants' Response to D

Claims 1 and 24 are amended to state that "probes" are "fluorescently labelled probes", which introduces basis for "monitored by fluorescence" (and also deals with the scope issues (below) that concern the issue of "which component" of the reaction is fluorescently labeled).

E. Claim 2 recites the limitation "**the** absolute CN of NucSeqII" (Item 24 of the Action). There is allegedly insufficient antecedent basis for this limitation which, in turn, is said to render the monitoring of the amplification indefinite. It is unclear to the Examiner where one obtains "the absolute CN of NucSeqII" in order to use the claimed invention.

Applicants' Response to E

Please note that Claim 2 had already been amended the in prior response to depend from Claim 18. Claim 18 depends from claim 17 instead of claim 1, so this ground should be reconsidered since there is proper antecedent basis, for the following reasons:

(1) Claim 17 introduces "cells" as antecedent basis for "absolute CN…*per cell*" in claim 18.

(2) Claim 18 introduces "an absolute CN" (per cell) as an antecedent basis for "absolute CN" in claim 2.

Applicants believe that this rejection may have been made in error, with the Examiner overlooking the prior amendments. Given the complexity of the claims, this is understandable and its withdrawal is respectfully requested.

F. Claims 1, 3-5,8, 11, 12, and 17 allegedly omit essential structural cooperative relationships of elements (item 25 of Action): The Examiner asks several questions as a reflection of the lack of clarity of various "relationships". The questioning in the Action goes into

> how a ratio of weight/vol concentrations of Conc-I_{SCI} to Conc-II_{SCII} in the formula of claim 1 can yield a relative CN. Copy Number is an expression of a number of things, the number of copies a sequence, and is not an expression of weight. If "Conc" were limited to molarity which is a measure of the number of moles or molecules where the molecule can be a sequence) per volume, then the formula can yield a relative CN. However no such limitation is found in the claims.

The Examiner indicates that he could find no definition in the specification limiting "Conc" to a measure of the number of "things" per volume.

G. Claims 24, 2, 6, 7, 9, 10, and 13-16 (Item 26 in Action) allegedly omit an essential structural cooperative relationships of elements. This is the same issue raised in (F) above but but relates instead to claim 24.

Applicants Response to F and G

The amendment introducing "relative CN" into claim 1 (and 24) should overcome this rejection since the only "CN" in the claim now is the "relative CN".

Since the concentration is measured by the amount of fluorescence that is formed during the amplification reaction, which is a consequence of hybridized, fluorescently labelled probes and since only one probe can hybridize per amplification product, concentration can not be expressed as wt/vol, moles/vol or molecules/vol. Nevertheless the concentration is reflected by the measurement of fluorescence and the "relative CN" can still be calculated.

While Applicants do not believe it to be necessary, If the Examiner is of the mind that that amending "concentration" to "amount" or "amount of fluorescence" will solve this matter, Applicants can amend the claims accordingly, but refrain from doing so now for the reasons stated.

IV. NEW REJECTIONS UNDER 35 USC § 112 FIRST PARAGRAPH (Enablement)

Claims 1-24 were rejected (Item 27 of Action) for lack of enablement because the specification, while being enabling for detection using probes with both quenchers and fluorophore, allegedly does not enable any person skilled in the art to use the invention commensurate in scope with these claims.

In the Office's *Wands* Analysis different points are raised under the various criteria, and some of these will be discussed below with each point followed by Applicants' remarks.

The nature of the invention/breadth of claims

The Action states that claims 1-24 broadly recite "any fluorescent method of monitoring" without any limitation on *what is fluorescing* and any expression of concentration. However, the art allegedly teaches the use of molar concentration to determine copy numbers and relative copy numbers (citing Ginzinger *et al.*(2000), Ginzinger *et al.*(2002), Zhang et al. (1997). and Zhang *et al.* (1997). Furthermore, each of these teachings allegedly states clearly what the fluorescent moiety is and how the assay format leads to a fluorescence measurement that is related to copy number. These references also go into detail on the amplification technique used how those techniques are suited to determining copy number.

Applicants' Response

As amended, and as discussed above, determining the "relative CN" of the present claims does not necessitate use of a molar concentration. First, "what is fluorescing" is rectified by amendment ("fluorescently labeled probe"). The fluorescently labelled probe may comprise any fluorescent compound known in the art which is suitable for detection in an amplification reaction. Using the concentration, determined by the fluorescence intensity, the relative CN can be calculated. The absolute CN may be calculated starting from the relative CN, if one multiplies by the absolute number of copies of the control sequence (NucSeqII) per cell. See claim 18 and its dependent claim, claim 2).

Working Examples

The specification provides two working examples but **only one method of determining copy number using:** (1) a probe labeled with a quencher and fluorophore, (2) amplification by RT-PCR, and (3) standard curves constructed using known copy numbers of controls per set volume, a very specific expression of concentration, **even more specific than molar concentration.**

Applicants' Response

The working examples are believed to be adequately illustrative when considering the amended claims.

Guidance in the Specification

The specification allegedly provides no evidence that methods other than a probe with a quencher and fluorophore can be used successfully.

Applicants' Response

Applicants respectfully traverse this position. It is believed that the person skilled in the art will appreciate and know how to perform the claimed fluorescence-based method in light of the claims as amended, the guidance and the high level of skill in the art. If a quencher is an absolute requirement for practice of the method, that is well-known to the skilled artisan.

It is well settled that

> ...the disclosure of an application embraces not only what is expressly set forth in words or drawing, but what could be understood by persons skilled in the art. As was said in *Webster Loom Co. v. Higgins et al.*, 105 US 580, 586, the applicant "may begin at the point where his invention begins, and describe what he has made that is new and what is replaced of the old. That which is common and well known is as if it were written out in the patent***." *In re Chelowsky*, 108 U.S.P.Q. 321, 324 (C.C.P.A. 1956) quoted w/ approval in *In re Folkers*, 145 USPQ 390, 394 (C.C.P.A. 1965).

Claims are not expected to recite such factors that are well-known and would be considered obvious to one of ordinary skill in the art to whom the specification and claims are directed (In re Skrivan, 166 U.S.P.Q. 85 (C.C.P.A. 1970).

In view of the foregoing, the Office's position of nonenablement based on the absence of recitation of a quencher in claim 1 (and 24) is not correct, so no reliance may be placed on this position for the current rejection.

The unpredictability of the art and the state of the prior art

The Action states that determining copy number is not a simple task, citing Ginzinger *et al.* (2002)). The Office also puts weight on post-filing art that allegedly supports the Office's view on the unpredictability of this field. Specifically, the Office cites Bustin *et al.* (2005):

> Worryingly, the extent of the unreliability of quantitative RT-PCR data [including copy number],and its effect on their biological validity, is still not widely appreciated or acknowledged.

(p 597, 2nd col., 2nd sentence). Bustin *et al.* elaborates:

> The principle of quantification is straightforward ....In practice, the relationship between target copy number and detection is not as clear-cut. First, reproducible quantification of any low abundance target «1000 copies) is problematic due to the inherent limitation of PCR amplification of small amounts of template ... Secondly, since many biological samples contain inhibitors of the RT and/or the PCR step ... Thirdly, it is essential to apply a normalization strategy to control for the amount of starting material, variation of amplification efficiencies and differences between samples"

(p. 599, and the 1st paragraph in the *Normalization* section).

Applicants' Response to Three Issues Raised

1. Reliability

Admittedly, there may be a certain degree of unreliability of quantitative RT-PCR data and its effect on biological validity. However, the problem of reproducible quantification of a low abundance target (<1000 copies) according to Bustin et al. (2005) seems rather minor.

For example Thorne et al. (2007) Diagn Mol Pathol 16:73-80) discloses the possibility of measuring 8 - 800,000 copies of CMV genomes per reaction. Yang et al. (2007) J. Agric. Food Chem. 55:15-24) disclose a detection limit of 20 copies for different GM maizes. Copies of both these papers are provided. Reischer et al. (2007) Lett Appl Microbiol 44:351-6, reports qualitative and quantitative detection limits of the PCR assay of 6 and 30 marker copies, respectively (abstract is provided).

Interestingly, in a paper by Nolan, Hands and Bustin (2006) Nature Protocols 1:1559-82 (copy provided), the same "Bustin group" points out the concerns about the reliability issues with RT-PCR ("the reverse transcription reaction is not standardized,

hence can be very variable samples, single cells, tissue culture cells…"). They disclose how to solve eventual problems linked to performing RT- PCR (See abstract).

This is exactly the point of strength of the present invention: use of a multiple standard to enhance the efficiency of the reaction and guarantee excellent reproducibility. Applicants emphasize that this concern of the Bustin group does not apply to relative quantification of one gene (or gene product) against another. Indeed the concerns voiced by the Bustin group are relevant only when NucSeqI and/or NucSeqII are RNA sequences, because, in the case of DNA sequences, reverse transcription is not applicable.

2. Many Biological Samples Contain Inhibitors of RT and/or the PCR step,

The Bustin group itself provides a method to test whether inhibitors are present in a sample:

> … it is crucial to assess the presence of any inhibitors of polymerase activity in RT and PCR. This is most easily achieved by running a reference RT-PCR assay, to which sample RNA is added, and measuring shifts in Ct (Smith et al, 2003)."

3. Applying "a normalization strategy to control for the amount of starting material, variation of amplification efficiencies and differences between samples"

The present invention in particular focuses on solving the above problem and provides a reliable normalization strategy, by using an "internal standard gene" which, in some claimed embodiments, occurs with a known copy number per cell. In this way, the invention as claimed overcomes this problem relating to reliability of quantitative RT-PCR data.

Quantity of Experimentation

The Action provides a string of reasons why the quantity of experimentation needed to practice the invention is "extremely large : …significant number of parameters …to be studied to encompass the claims as written including the labeling of anyone or some combination of nucleotides, primers, probes, polymerases, and any other reagent used… the choice of fluorescent label(s); the exploration of endogenous fluorescence…, …homogeneous or heterogeneous format; …competitive or non-competitive format. Choice and optimization of these parameters and formats would require a large quantity of experimentation. This would require considerable inventive effort, upon effective reduction to practice, not providing any guarantee of success.

Applicants' Response

Applicants believe that the quantity of experimentation needed to carry out the invention as defined by the amended claims in view of the specification and state of the art is not large and certainly not inventive or undue. These are simply conclusory

statements made by the Examiner that Applicants believe exaggerate the issue and seem to completely ignore the advanced state of the art as it impacts indicated parameters of such assays. Applicants again respectfully remind the Examiner of the case law cited above that it is not the function of claims to recite factors that are known in the art.

The amendment of the present claims to recite that it is the probe that is fluorescently labeled removes a great deal of "unknown scope" from the above considerations. Moreover, a skilled artisan will readily know how carry out amplification reactions using a fluorescently labelled probe in any format, whether competitive or non-competitive. This will require no more than routine experimentation and optimization, as would be expected in most assays of this type. The specification enables one to make and use the invention to the full scope of the currently amended claims without with a strong expectation of success.

Guidance in the Specification

The Office alleges that no evidence is provided that monitoring fluorescence of **any** component would be feasible to determine relative copy number, *e.g.*, the specification does not disclose how labeling a polymerase with a fluorophore can be used,

Applicants' Response

This concern is taken care of by the present amendments that specifically recite that it is the probe that is fluorescently labeled. The specification provides evidence that monitoring fluorescence of a fluorescently labelled probe in an amplification reaction is feasible for determining the relative copy number (as now recited) of a nucleotide sequence in a sample as presently claimed.

V. CONCLUSION

In view of the amendments to the claims and the foregoing remarks, Applicants believe that they have overcome or mooted the various grounds for rejection. Reconsideration, withdrawal of the rejections and allowance of the amended claims are respectfully requested.

The Examiner is respectfully requested to contact the undersigned at (202) 628-5197 if any clarification is required or if further discussion will assist in continued examination of this application

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Respectfully submitted,

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